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# Infection with parasitic nematodes confounds vaccination efficacy

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**Abstract**

T helper (Th) cells produce signature cytokine patterns, induced largely by intracellular versus extracellular pathogens that provide the cellular and molecular basis for counter regulatory expression of protective immunity during concurrent infections. The production of IL-12 and IFN-γ, for example, resulting from exposure to many bacterial, viral, and protozoan pathogens is responsible for Th1-derived protective responses that also can inhibit development of Th2-cells expressing IL-4-dependent immunity to extracellular helminth parasites and vice versa. In a similar manner, concurrent helminth infection alters optimal vaccine-induced responses in humans and livestock; however, the consequences of this condition have not been adequately studied especially in the context of a challenge infection following vaccination. Demands for new and effective vaccines to control chronic and emerging diseases, and the need for rapid deployment of vaccines for bio security concerns requires a systematic evaluation of confounding factors that limit vaccine efficacy. One common albeit overlooked confounder is the presence of gastrointestinal nematode parasites in populations of humans and livestock targeted for vaccination. This is particularly important in areas of the world were helminth infections are prevalent, but the interplay between parasites and emerging diseases that can be transmitted worldwide make this a global issue. In addition, it is not clear if the epidemic in allergic disease in industrialized countries substitutes for geohelminth infection to interfere with effective vaccination regimens. This presentation will focus on recent vaccination studies in mice experimentally infected with *Heligmosomoides polygyrus* to model the condition of gastrointestinal parasite infestation in mammalian populations targeted for vaccination. In addition, a large animal vaccination and challenge model against *Mycoplasma hyopneumonia* in swine exposed to *Ascaris suum* will provide a specific example of the need for further work in this area, and for controlled field studies to assess the impact of other similar scenarios.


*Keywords:* Th2-associated immunity; Immune modulating parasitic infection; Cytokine gene expression; Protective immunity

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## 1. Introduction

Vaccine development has driven the field of immunology since it incorporates the selection and presentation of benign antigens or attenuated pathogens to stimulate an acquired protective response. Vaccine strategies, in turn, have advanced with molecular techniques that define target antigens critical to the survival and pathogenicity of the infectious agent, and delivery systems that have become more sophisticated in the activation of host effector mechanisms appropriate to control infection. For example, it is now possible to develop recombinant vaccine platforms selected to preferentially stimulate CD4+ or CD8+ responses and effector mechanisms suitable for effective protection. Vaccination has proven to be the most cost effective and efficient procedure for disease management. The need to control chronic and emerging diseases and bio security concerns stimulate demand for new vaccines. Experimental rodent studies that followed shortly after the seminal description of Th1 and Th2 polarization *in vitro* by Mosmann et al. (1986) showed that parasitic infection could skew the immune response to non-parasite antigens (Kullberg et al., 1992). These observations supported later studies in humans that showed the consequences of concurrent helminth infection with schistosomes (Sabin et al., 1996) and geohelminths (Cooper et al., 2000; Elias et al., 2001) on vaccine efficiency, and remain relevant to modern vaccination strategies to manage chronic infectious diseases like tuberculosis, HIV, and malaria (Borkow and Bentwich, 2000).

Vaccine efficacy is equally important to the hundreds of veterinary vaccines in circulation worldwide because approval is largely based on safety and less on efficacy. Concomitant parasitic infection is a major factor in the free-range and organic farm management of livestock, as well as under more intensive management in confinement and on feed-lots. There is also the concern for the many zoonotic infections that are threats to bio security and worrisome because of the need for development of effective vaccines to limit contagious disease. An underlying helminth infection can also interfere with the accurate diagnosis of infectious status as demonstrated recently in a model of co-infection of cattle with *Fasciola hepatica* and bovine tuberculosis (Flynn et al., 2007). These concepts are generally evaluated first in experimental rodent models to demonstrate specific cellular and molecular components that affect vaccination efficacy. It is critical, however, to further develop vaccination and challenge studies in the relevant host species and to extend the work to field trials in order to ensure the success of vaccination in an integrated strategy to control disease. The use of multiple species, including large animal models, to test vaccination procedures against emerging and zoonotic diseases will help to control dangerous infections and provide comparative models to develop vaccines against infectious agents restricted from testing in humans. This presentation describes an effective rodent model to examine general features of nematode parasite infection on vaccine efficacy, and the extrapolation of some of these findings to a swine model of vaccination against a common respiratory pathogen.

## 2. Mouse model

### 2.1. Development of a robust parasite/rodent model for vaccination studies

Mice infected with *Heligmosomoides polygyrus* have several advantages as a model to test the hypothesis that gastrointestinal parasitic infection can affect vaccine efficacy. (1) The parasite naturally infects mice and thereby embodies the co-evolutionary factors driving adaptations critical to both host and parasite survival. The third-stage larva (L3) of this trichostrongyle parasite infects the host orally, rapidly invades the duodenum to encyst in a sub mucosal region of the small intestine, and adults emerge into the lumen 8 days after inoculation. (2) The infection is chronic (common to most geohelminth infections of humans and livestock) and lasts for weeks to months depending on the genetics of the strain of mice used. (3) Anthelmintic treatment cures the infection to induce acquired immunity and a strong protective response that is CD4+ T cell-dependent. (4) The molecular and cellular aspects of protective immunity and immune modulating components of the infection in mice are well-defined (Finkelman et al., 2004; Liu et al., 2004).

### 2.2. Immune characteristics induced by infection with H. polygyrus

There are features of a *H. polygyrus*-induced immune response postulated to obstruct optimal development of an effective vaccination against pathogens that require Th1-derived immunity. The response to *H. polygyrus* is primarily Th2-polarized without evidence of an underlying Th1 response (Liu et al., 2004). Thus, there is expression of IL-3, IL-4, IL-5, IL-9, and IL-13, with little detectable IFN-γ, and the development of eosinophilia, basophilia, mucosal mast cell and goblet cell hyperplasia. The antibody response

to parasite antigens is primarily IgG1 and IgE; subclasses that contribute to passive immunity to infection in rodent pups (Harris et al., 2006), but not appreciably to the control of worm infection in adult mice. It is notable, however, that the predominant IgG1 and IgE antibody response following infection extends to concomitant exposure to non-parasite antigens (Liu et al., 2005). This has consequences for vaccines when pathogen specific IgG2a, IgG2b, and IgG2c antibodies that can appropriately neutralize infectious agents via complement fixation and opsonization appear re-directed to expression of inappropriate IgG1 and IgE antibody isotypes.

The cytokine pattern induced by *H. polygyrus* also has consequences for cellular and physiological events that modulate immune function; especially at mucosal surfaces. The rapid entry of L3 into the submucosa region of the small intestine at day 4 after a secondary inoculation induces neutrophils and alternatively activated macrophages (AAMΦs) around the encysted parasite, and a band of CD4+ T cells and CD11c+ dendritic cells surrounds the macrophages. The macrophages express markers characteristic of AAMΦs including IL4R, CD206, and arginase-1, but not iNOS; and their accumulation is dependent on memory CD4+ T cells (Anthony et al., 2006, in press). Treatment of infected mice with chlodronate-loaded liposomes to deplete macrophages or an arginase inhibitor to alter AAMΦs function results in increased larval mobility, decreased stress-induced larval cytochrome oxidase expression, and reduced adult worm expulsion. These data suggest that AAMΦs contribute to protective immunity. Concomitant infection reveals an important functional aspect of AAMΦ development since mice infected with the cestode *Taena crassiceps* and subsequently co-infected with *Leishmania major* and *Leishmania mexicana* express a Th2-type response without down regulation of IFN-γ. Nevertheless, the protective response to *Leishmania* was blocked and disease was enhanced (Rodriguez-Sosa et al., 2006). Chen et al. (2006) demonstrated a similar phenomenon where infection of mice with *H. polygyrus* exacerbated colitis induced by exposure to *Citrobacter rodentium*, a natural bacterial infection of rodents. The cell population responsible is lamina propria dendritic cells acting through an IL-10-dependent suppression of host resistance. The normal Toll-like receptor 4 (TLR4)-induced response of mucosal T cells is altered following infection with *H. polygyrus* to produce TGF-β (Ince et al., 2006), and CD8+ lamina propria T cells induced by *H. polygyrus* inhibited T cell proliferation and experimentally induced colitis (Metwali et al., 2006). Prior infection with *H. polygyrus* has been shown to down regulate allergic symptoms and peanut-specific IgE in a mouse model of peanut allergy. Treatment of mice with neutralizing antibody to IL-10 eliminated the protective effect of *H. polygyrus* infection, suggesting that helminth infection induces immune regulatory cytokines that can minimize allergic responses (Bashir et al., 2002). In addition, a regulatory CD4+/CD25+ T cell population reduced responses to non-parasite allergens in the lungs of *H. polygyrus* infected mice. These observations indicate local intestinal development of T regulatory cells with diverse function that disseminate to other mucosal sites (Wilson et al., 2005). Parasite-induced IL-4 and IL-13 initiate stereotypical changes that are IL-4 receptor alpha chain-linked and STAT6-dependent (Finkelman et al., 2004). These include changes in epithelial cell function contributing to adult worm expulsion by a ‘‘weep and sweep’’ protective response (Shea-Donohue and Urban, 2004). There are programmed changes in sodium-linked glucose transporters and markers of intestinal permeability that alter the microenvironment surrounding the worm, but may also alter the absorption and presentation of non-parasite antigens delivered to mucosal sites in the intestine in ways not fully explored.

### 2.3. *H. polygyrus* affecting vaccination outcome

The utility of the *H. polygyrus* infection and vaccination model was recently evaluated in the context of immunization against rodent *Plasmodium* species. Su et al. (2006) demonstrated that infection with *H. polygyrus* reduced the normally strong immunity to *Plasmodium chabaudi* following immunization with a crude blood-stage antigen. The levels of malaria-specific antibody were significantly lower, and spleen cells from immunized nematode-infected mice produced lower levels of IFN-γ, but more IL-4, IL-13, IL-10, and TGF-β. Anthelmintic treatment before anti-malarial immunization, but not after, restored the protective immunity to malaria challenge. In another malaria model, Noland et al. (2005) observed that *Echinostoma caproni* exacerbated *P. yoelii* malaria in a concomitant infection, and infection with either *E. caproni* or *H. polygyrus* altered the titer and antibody class distribution in mice immunized with a Pfs25 antigen transmission blocking DNA vaccine (Noland et al., 2007). This has particular significance because blockage of gametocyte transmission to the arthropod vector is dependent on the ingestion of high affinity antibodies and specific isotypes during blood feeding on immunized hosts that can prevent expansion of the

parasite in the mosquito. The speculation is that infection with *H. polygyrus* alters antibody responses to *Plasmodium* antigens by shifting the isotype pattern and blocking pathways dependent on antigen presenting cells expressing IL-12, and implicating systemic effects of a strictly enteric helminth infection.

Another recent example of interference in vaccination comes from studies describing the Th2 polarizing effect of *H. polygyrus* on a mucosal vaccine (Iweala et al., 2007). In this system, a novel OVA-expressing oral *Salmonella* vaccine (*Salmonella*-OVA) that normally induces a Th1 biased systemic OVA specific response was given in the presence of infection with *H. polygyrus*. The Th1 dependent serum OVA specific IgG2c response was delayed and reduced, while serum OVA specific IgG1 response was enhanced. The infection also reduced the production of IFN-γ by CD4+ splenocytes isolated from vaccinated mice and re-stimulated *in vitro* with OVA, while enhancing the production of IL-13 and IL-10 following stimulation with anti-CD3. Thus, concomitant infection with *H. polygyrus* significantly alters the immune response to oral vaccination with a non-parasite antigen presented by an attenuated enteric bacterium. Altered antigen uptake into localized lymphoid follicles and processing by dendritic cells affected by factors from the worm are possible cellular mechanisms behind this altered immune response.

## 3. Swine model

### 3.1. Immune characteristics induced by infection with Ascaris suum

Infection with *A. suum* is common in pigs worldwide with migrating larvae producing focal liver lesions and eosinophilic pneumonitis in both humans and pigs. There is a predominant Th2 response based on a cytokine gene expression pattern in tissues draining sites of infection, an associated expulsion of fourth-stage larvae (L4) from the small intestine, and a localized mast cell-dependent immediate type hypersensitivity response to parasite antigens (Dawson et al., 2005). Unlike the *H. polygyrus* mouse model, however, there is a low-level Th1 and anti-inflammatory cytokine gene expression, and an induced bronchial alveolar exudate around the time larvae transverse the lungs that is largely eosinophilic. A prototypical immune and physiological response to infection including increased small intestinal smooth muscle contractility and reduced epithelial cell glucose transport is common to the two infection models (Dawson et al., 2005). A proportion of the cells in the bronchial alveolar lavage at 14 and 21 days after inoculation is alveolar macrophages that have properties of AAMΦs including gene expression of the mannose receptor, high levels of arginase-1, and low iNOS (Solano-Aguilar et al., 2007). In addition, alveolar macrophages from uninfected pigs express similar levels of gene expression for AAMΦs markers when cultured *in vitro* with recombinant porcine IL-4 (Dawson et al., unpublished results). Notable is the functional aspects of alveolar macrophages from *A. suum*-infected pigs because phagocytosis of opsonized and formalin-fixed *Staphylococcus aureus in vitro* was significantly decreased, but production of reactive oxygen species, including hydrogen peroxide and super oxide anion, was increased (Solano-Aguilar et al., 2007). This functional phenotype appears better prepared to respond to extracellular but not intracellular pathogens.

### 3.2. A. suum affecting vaccination outcome

*Mycoplasma hyopneumonia* commonly causes porcine enzootic pneumonia in production swine with high morbidity. Vaccination against infection is cost effective and benefits swine health, growth, and general welfare. Co-infection of pigs with *M. hyopneumonia* and *A. suum* is likely in most swine producing facilities worldwide, and provides an excellent model to test the impact of persistent helminth infection on vaccine efficacy and the consequences of a challenge infection. Steenhard et al. (2007) trickle-infected pigs with 25 *A. suum* eggs/kg/day) twice per week throughout the experiment, immunized the pigs with a killed-*M. hyopneumonia* vaccine 3 weeks after the first inoculation, and subsequently challenged with live *M. hyopneumonia* strains 4 weeks after vaccination. The antibody response of vaccinated pigs not exposed to worms showed a serum-conversion of 100% at 3 weeks after vaccination that persisted throughout the study. In contrast, only 33% of vaccinated pigs infected with *A. suum* had a significant antibody response to *M. hyopneumonia* after 3 weeks, and the final serum conversion level did not exceed 78% of those vaccinated without parasite exposure. In addition, vaccinated pigs exposed to *A. suum* had more lung pathology following the challenge infection with *M. hyopneumonia* compared to vaccinated pigs without worms. The results show that *A. suum* significantly compromised the efficacy of vaccination against *M. hyopneumonia*. Since the mechanism of protective

immunity to *M. hyopneumonia* includes both humoral and cellular components, the impact of infection with *A. suum* may be through altered alveolar macrophage function or a reduction in the level and class of anti-*M. hyopneumonia* antibodies most appropriate to control infection. It appears clear that more attention to this area of research is needed to outline possible mechanisms, and to predict and control for desirable vaccination outcomes.

## 4. Discussion

The platforms for modern vaccines range from plasmid DNA to plant-based expressed systems along with the more conventionally produced live or killed vaccines for acute viral or bacterial diseases. Equally sophisticated delivery systems include designer adjuvant formulations that utilize TLR ligands and other innate immune activators. The goal is to provide rational vaccine design and delivery that safely induces quantitatively and qualitatively improved cell mediated and humoral immune responses appropriate to protection from a challenge infection. Confounders are numerous and differ when the target is prophylactic vaccination of an individual versus reduced risk of transmission of highly contagious agents. Individual factors of age, sex, diet, health status, and genetic composition become unmanageable when population or herd immunizations are required. Threats from infectious epidemics, dangers of emerging and zoonotic diseases, and security from the threat of select agent exposures, however, must consider a global setting where co-infection of humans as primary targets and animals as reservoirs are likely to carry parasitic helminths. The co-evolutionary development of parasitism is inherently an immune modulating interaction with consequences for specific vaccination requirements. Rodent models are generally useful to address specific mechanisms of immune function and proof of principle concepts of complex molecular and cellular interactions. The demonstration that mice infected with *H. polygyrus* reduce *Plasmodium* vaccine efficacy of both a crude blood-stage antigen vaccine (Su et al., 2006) as well as a DNA-based transmission blocking vaccine (Noland et al., 2007) indicates that a range of vaccination strategies are at risk. Notable, however, is that anthelmintic treatment prior to vaccination successfully reversed worm interference with vaccination and the protective response. Anthelmintic clearance of worm infection is a routine and generally effective procedure in both humans and livestock, and should be considered as a component in an integrated strategy that includes vaccination. Although the incidence of worm infection in humans in industrialized countries is generally low, there are strong parallels between the immune response to worms and expression of allergic responses; which are a major health concern for a large segment of the population. Thus, conditions that induce a localized allergic environment in the lungs contribute to reduced clearance of viral infection and may contribute to conditions that limit vaccination efficacy as well (Hogan et al., 1998).

The study of vaccination against large animal infectious diseases inherently optimizes animal health, and the production of more efficient and safe agricultural products. This task has become more definitive by the increase in genomic information in major livestock species and the development of functional databases to generate species comparative immunological tools such as that shown in the Pig Nutrition and Immunology (PIN) database http://www.ars.usda.gov/Services/docs.htm?docid=6065.

Parasite control is an important component of any livestock management scheme for enhancing production. It needs to be considered for vaccination protocols that protect against production, zoonotic, and emerging diseases. Large animals also provide more relevant modeling of both zoonotic infections and those pathogen interactions that correspond more directly to human diseases; as is the case with the interaction between *Mycoplasma* and *Ascaris* species as common pathogens in humans. Steenhard et al. (2007) used a commercial *Mycoplasma* vaccine and low dose trickle infection with *A. suum* to simulate a scenario likely in most swine production facilities. The results confirmed the hypothesis that parasite infection reduced vaccination efficacy with negative consequences on protective immunity and increased pathology in the lungs. The reduced serum conversion and quantitative titers in the *Ascaris*-infected and vaccinated pigs is comparable to that observed in infected mice vaccinated with crude (Su et al., 2006), molecular (Noland et al., 2007), and mucosal vaccines (Iweala et al., 2007). Enhanced pulmonary pathology from a live *M. hyopneumonia* challenge may relate to altered alveolar macrophage function resulting from migration of *A. suum* larvae in the lung (Solano-Aguilar et al., 2007). Testing the concept further in a well-designed field trial is reasonable and approachable in several commercial production facilities not feasible with human population studies.

Many aspects of vaccine design and implementation are driven by advancing molecular technology and basic information of host/pathogen interactions that target

pathogen vulnerability and reduced host pathology. Experimental vaccine development under controlled conditions in the laboratory requires field testing to isolate important modulating factors. An underlying parasitic infection is a profound, albeit reversible, modifier of vaccine efficacy.

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